

FIG. 1A

EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNG  
GTSHNQREFMDRFTISVDKSTSTAYMQMNSLRAEDTAVYCARWRGLNYGFDVRYFD  
VWGQGILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL  
TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHHKPSNTKVDDKKVEPKSCDK  
THTCPPCPAPELLGGRMKQLEDKVEELLSKNYHLENEVARLKKLVGER

FIG. 1A

DIQMTQSPSSLSASVGDRVTITCRASQDINNLYNWYQQKPKAPKLLNYTSTLHSGVP  
SRFSGSGGTDTYLTISSLQPEDFATYCCQGNLPTTFGQGTKVEIKRTVAAPSVFIFPP  
SDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSLST  
LTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

FIG. 1B

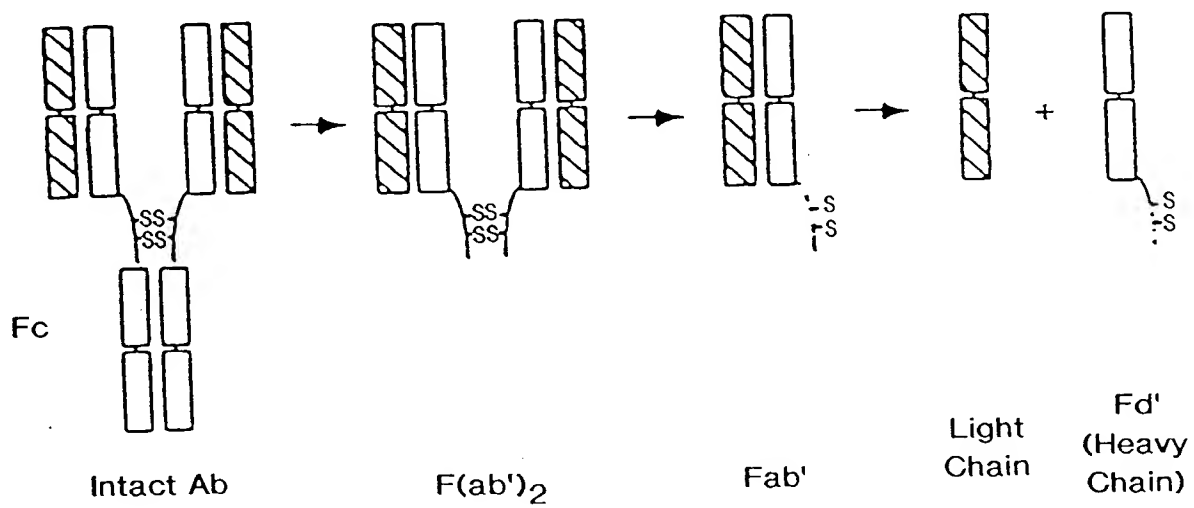


FIG. 2A

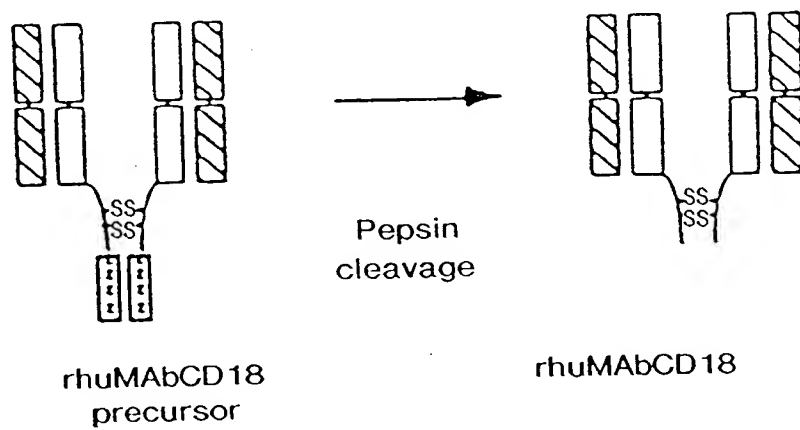


FIG. 2B

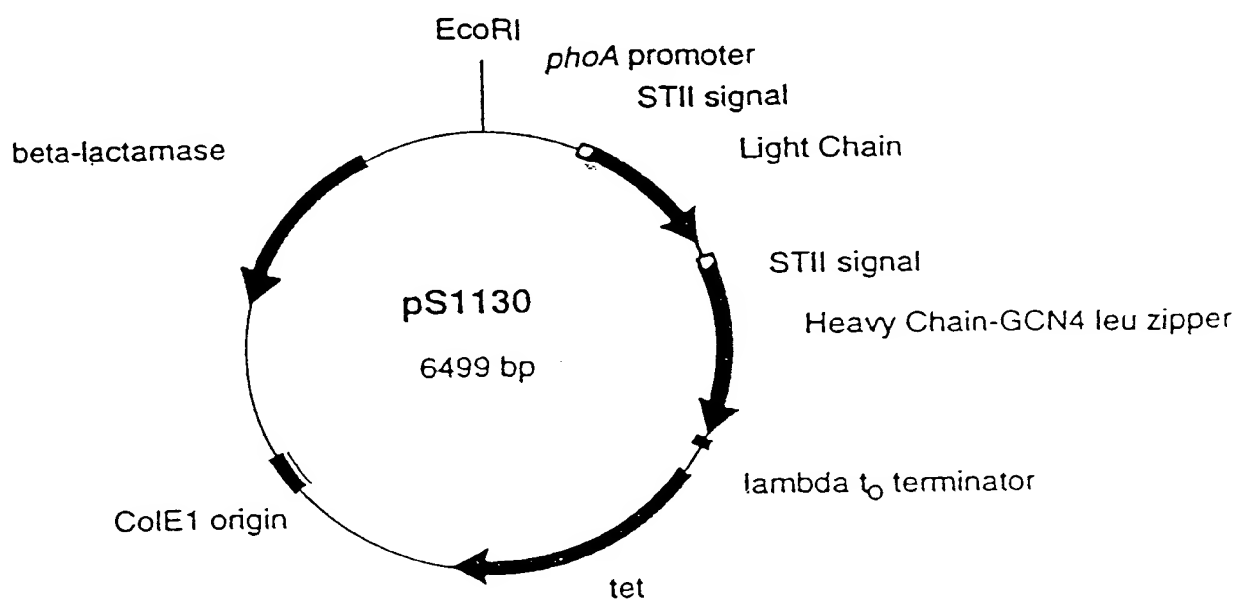


FIG. 3

1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA  
61 GTTGTATTATT AAGCTTTGGA GATTATCGTC ACTGCAATGC TTCGCAATAT GGCGCAAAAT  
121 GACCAACAGC GGTGTATTGA TCAGGTAGAG GGGGCGCTGT ACGAGGTAAA GCCCGATGCC  
181 AGCATTCCCTG ACGACGATAC GGAGCTGCTG CGCGATTACG TAAAGAAGTT ATTGAAGCAT  
241 CCTCGTCAGT AAAAAGTTAA TCTTTTCAAC AGCTGTCATA AAGTTGTCAC GGCCGAGACT  
301 TATAGTCGCT TTGTTTTTAT TTTTAAATGT ATTTGTAAC AGAATTCGAG CTCGCCGGGG  
361 ATCCTCTAGA GGTTGAGGTG ATTTT ATG AAA AAG AAT ATC GCA TTT CTT CTT  
-23 M K K N I A F L L  
413 GCA TCT ATG TTC GTT TTT TCT ATT GCT ACA AAC GCG TAC GCT GAT ATC  
-14 A S M F V F S I A T N A Y A D I  
461 CAG ATG ACC CAG TCC CCG AGC TCC CTG TCC GCC TCT GTG GGC GAT AGG  
3 Q M T Q S P S S L S A S V G D R  
509 GTC ACC ATC ACC TGT CGT GCC AGT CAG GAC ATC AAC AAT TAT CTG AAC  
19 V T I T C R A S Q D I N N Y L N  
557 TGG TAT CAA CAG AAA CCA GGA AAA GCT CCG AAA CTA CTG ATT TAC TAT  
35 W Y Q Q K P G K A P K L L I Y Y  
605 ACC TCC ACC CTC CAC TCT GGA GTC CCT TCT CGC TTC TCT GGT TCT GGT  
51 T S T L H S G V P S R F S G S G  
653 TCT GGG ACG GAT TAC ACT CTG ACC ATC AGC AGT CTG CAA CCG GAG GAC  
67 S G T D Y T L T I S S L Q P E D  
701 TTC GCA ACT TAT TAC TGT CAG CAA GGT AAT ACT CTG CCG CCG ACG TTC  
83 F A T Y Y C Q Q G N T L P P T F  
749 GGA CAG GGC ACG AAG GTG GAG ATC AAA CGA ACT GTG GCT GCA CCA TCT  
99 G Q G T K V E I K R T V A A P S  
797 GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC  
115 V F I F P P S D E Q L K S G T A  
845 TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA  
131 S V V C L L N N F Y P R E A K V  
893 CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT  
147 Q W K V D N A L Q S G N S Q E S  
941 GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC  
163 V T E Q D S K D S T Y S L S S T  
989 CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC  
179 L T L S K A D Y E K H K V Y A C  
1037 GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC  
195 E V T H Q G L S S P V T K S F N  
1085 AGG GGA GAG TGT TAA G CTGATCCTCT ACGCCGACG CATCGTGGCG  
211 R G E C

FIG. 4A

1131 CTAGTACGCA AGTTCACGTA AAAACGGTAT CTAGAGGTTG AGGTGATTTT ATG AAA  
-23 M K

1187 AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT TTT TCT ATT GCT  
-21 K N I A F L L A S M F V F S I A

1235 ACA AAC GCG TAC GCT GAG GTT CAG CTG GTG GAG TCT GGC GGT GGC CTG  
-5 T N A Y A E V Q L V E S G G G L

1283 GTG CAG CCA GGG GGC TCA CTC CGT TTG TCC TGT GCA ACT TCT GGC TAC  
12 V Q P G G S L R L S C A T S G Y

1331 ACC TTT ACC GAA TAC ACT ATG CAC TGG ATG CGT CAG GCC CCG GGT AAG  
28 T F T E Y T M H W M R Q A P G K

1379 GGC CTG GAA TGG GTT GCA GGG ATT AAT CCT AAA AAC GGT GGT ACC AGC  
44 G L E W V A G I N P K N G G T S

1427 CAC AAC CAG AGG TTC ATG GAC CGT TTC ACT ATA AGC GTA GAT AAA TCC  
60 H N Q R F M D R F T I S V D K S

1475 ACC AGT ACA GCC TAC ATG CAA ATG AAC AGC CTG CGT GCT GAG GAC ACT  
76 T S T A Y M Q M N S L R A E D T

1523 GCC GTC TAT TAT TGT GCT AGA TGG CGA GGC CTG AAC TAC GGC TTT GAC  
92 A V Y Y C A R W R G L N Y G F D

1571 GTC CGT TAT TTT GAC GTC TGG GGT CAA GGA ACC CTG GTC ACC GTC TCC  
108 V R Y F D V W G Q G T L V T V S

1619 TCG GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC  
124 S A S T K G P S V F P L A P S S

1667 AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC  
140 K S T S G G T A A L G C L V K D

1715 TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC  
156 Y F P E P V T V S W N S G A L T

1763 AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC  
172 S G V H T F P A V L Q S S G L Y

1811 TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG  
188 S L S S V V T V P S S S L G T Q

1859 ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTC GAC  
204 T Y I C N V N H K P S N T K V D

1907 AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCG CCG  
220 K K V E P K S C D K T H T C P P

1955 TGC CCA GCA CCA GAA CTG CTG GGC GGC CGC ATG AAA CAG CTA GAG GAC  
236 C P A P E L L G G R M K Q L E D

2003 AAG GTC GAA GAG CTA CTC TCC AAG AAC TAC CAC CTA GAG AAT GAA GTG  
252 K V E E L L S K N Y H L E N E V

2051 GCA AGA CTC AAA AAG CTT GTC GGG GAG CGC TAA GCATGCG ACGGCCCTAG  
268 A R L K K L V G E R

2101 AGTCCCTAAC GCTCGTTGCG CGCCGGGCGT TTTTATTGT TAA

FIG. 4B

<u>Strain</u>	<u>Genotype</u>
W3110	K-12 F <sup>-</sup> lambda <sup>-</sup> IN $\text{rmD-rmE1}$
↓	
1A2	W3110 $\Delta fhuA$
↓	
7C1	W3110 $\Delta fhuA \Delta phoA \Delta(\text{argF-lac})$
↓	
16C9	W3110 $\Delta fhuA \Delta phoA \Delta(\text{argF-lac}) deoC$
↓	
23E3	W3110 $\Delta fhuA \Delta phoA \Delta(\text{argF-lac}) deoC \Delta degP$
↓	
33B6	W3110 $\Delta fhuA \Delta phoA \Delta(\text{argF-lac}) deoC \Delta degP ilvG$
↓	
49B2	W3110 $\Delta fhuA \Delta phoA \Delta(\text{argF-lac}) deoC \Delta degP ilvG \Delta fucP$
↓	
49A5	W3110 $\Delta fhuA \Delta phoA \Delta(\text{argF-lac}) deoC \Delta degP ilvG \Delta fucP \Delta malE$

FIG. 5

VIAL FROM  
WORKING CELL BANK  
OR  
MASTER CELL BANK

PRIMARY INOCULUM  
SHAKE FLASK  
MEDIUM

SECONDARY INOCULUM,  
SECONDARY MEDIUM

PRODUCTION VESSEL,  
PRODUCTION MEDIUM

HARVEST BY CENTRIFUGATION

FREEZING OF CELLS

14-18 hours  
temperature controlled

7-17 hours  
temperature and pH controlled  
transferred at 10-25 OD<sub>550</sub>

temperature and pH controlled  
controlled nutrient feeds  
harvested at 60-84 hours

FIG. 6

### Frozen Cell Pellets



### Homogenization and Centrifugation



## Cation Exchange Chromatography



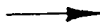
### Immobilized Pepsin Cleavage



### In line Charged Filter



## Filter Regeneration Pool

Digested  
Precursor

### Antibody Pool



## Cation Exchange Chromatography



## Hydrophobic Interaction Chromatography

FIG. 7